Design of multiplex assays for molecular subtyping of pathogens with the Luminex xMAP technology

Véronique Wuyts[1,2], Nancy Roosens [2], Sophie Bertrand [3], Kathleen Marchal [1,4], Sigrid De Keersmaecker [2]

(1) Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium
(2) Platform Biotechnology and Molecular Biology, Scientific Institute of Public Health, Brussels, Belgium
(3) Bacterial Diseases Division, Communicable and Infectious Diseases, Scientific Institute of Public Health, Brussels, Belgium
(4) Department of Plant Biotechnology and Bioinformatics, Ghent University (VIB), Gent, Belgium

Multiplex assays are a powerful tool for molecular subtyping of pathogens, which is crucial for rapid diagnosis, surveillance and identification and containment of outbreaks. The Luminex xMAP technology allows detection of up to 500 different analytes per sample in a high-throughput format through a liquid bead suspension array. Different types of Luminex assays can be envisaged. Each assay has its advantages and limitations, some of which are related to the inherent design of the primers and probes used. Software that predicts melting temperatures, hybridization structures and specificity for different types of oligonucleotides, and thereby simulating the multiplex assays *in silico* can facilitate the design of multiplex assays by reducing time and cost of experimentation.

We have compared two commercial software packages for the development and *in silico* simulation of a ligation dependent amplification (LDA) assay and a direct hybridization assay for the subtyping of a pathogen. The main findings, limitations and challenges will be discussed.